

AMENDMENTS TO THE CLAIMS

1. (Currently Amended) A process for purification of a heterologous protein of interest, comprising

(a) providing a fusion protein comprising said heterologous protein fused to a **carbohydrate binding module (CBM) (CBM)** intercepted by a proteolytic cleavage site,

(b) contacting said fusion protein with a functional protease fused to a CBM, at conditions facilitating proteolytic cleavage by said protease, to cleave the CBM from the heterologous protein of interest,

(c) contacting the solution of CBM-protease, free CBM and heterologous protein of interest to a polysaccharide matrix, under conditions where the CBM-protease and free CBM binds to said polysaccharide matrix and where the heterologous protein of interest is not retained on said polysaccharide matrix,

(d) separating the non-bound heterologous protein of interest from the polysaccharide matrix,

(e) washing the polysaccharide matrix with the bound CBM-protease and CBM, with one or more suitable aqueous solutions,

(f) eluting the CBM-protease from the matrix by adjusting conditions effecting the release of said CBM-protease off the matrix; and

(g) optionally reconditioning said eluted CBM-protease, to retain its affinity to said polysaccharide matrix, such that the reconditioned CBM-protease can be re-used for subsequent repetition of the process defined by steps (a)-(g),

wherein said CBMs are capable of binding reversibly to a polysaccharide matrix and being released from such matrix by non-denaturing elution conditions and do not bind substantially to insoluble cell-wall plant material.

2. (Previously Presented) The process of claim 1, wherein said protease fused to CBM is from the group of proteases consisting of enterokinase, tobacco etch virus (TEV) protease, factor X and thrombin.

3. (Original) The process of claim 2 wherein said protease is mammalian enterokinase (EK) or an enterokinase active part thereof.

4. (Original) The process of claim 3, wherein said EK comprises a bovine EK catalytic domain (EKc).

5. (Original) The process of claim 4, wherein said bovine EKc is encoded by the nucleic acid sequence shown as SEQ ID NO: 2.

6. (Original) The process of claim 1, wherein said protease fused to CBM and said heterologous protein fused to a CBM intercepted by a proteolytic cleavage site are obtained separately by a method for production and purification of a soluble heterologous fusion protein comprising a cellulose binding module (CBM), from transgenic plants or transgenic plant cells expressing said fusion protein, comprising the steps of:

(a) disrupting the transgenic plant material ;

(b) adding an extraction liquid to the plant material, thereby creating a mixture of soluble and insoluble plant material, so as to extract the soluble fusion protein from said disrupted plant material to the liquid phase to obtain a protein extract; (c) separating the insoluble plant material,

(c) comprising cell-wall material and solids, from said protein extract comprising said fusion protein of interest;

(d) contacting said protein extract to a polysaccharide matrix which binds to said fusion protein;

(e) washing the matrix with the bound fusion protein with one or more suitable aqueous solutions; and

(f) eluting the fusion protein from said polysaccharide matrix by adjusting conditions effecting the release of said fusion protein from the matrix, thereby obtaining the soluble heterologous fusion protein substantially purified.

7. (Original) The process of claim 6, wherein the separation step (c) comprises a method selected from expanded bed adsorption (EBA), packed mode chromatography, precipitation, filtration, centrifugation, or any combination thereof.

8. (Original) The process of claim 6 wherein affinity binding to said polysaccharide matrix in step (d) comprises a chromatography step.

9. (Original) The process of claim 6 wherein step(c) and (d) are performed simultaneously in a combined single step.

10. (Original) The method of claim 6, combining steps (c) and (d) in a process step comprising expanded bed adsorption with a polysaccharide matrix, as a measure for simultaneous separation of cell-wall material and solids from said protein extract and affinity binding of said CBM-fusion protein onto the polysaccharide matrix.

11. (Previously Presented) The process of claim 1, wherein said polysaccharide matrix comprises cellulose.

12. (Original) The process of claim 11, wherein said cellulose is a pharmaceutically compatible cellulose.

13. (Currently Amended) The process of claim 12, wherein said cellulose is ~~a~~^{AVICEL} AVICELTM.

14. (Previously Presented) The process of claim 1, wherein said reconditioning of the eluted CBM-protease involves neutralization, and/or removal from the CBM- protease eluent of agents that affect the release of CBM from said polysaccharide matrix.

15. (Original) The method of claim 14, wherein said reconditioning comprises neutralization or removal from the eluent of carbohydrates such as saccharides,

16. (Previously Presented) The method of claim 1 wherein said fusion protein comprising said heterologous protein of interest is expressed and retrieved from a transgenic plant or plant cell or by transient expression in a plant, plant tissue or a plant cell.

17. (Original) The method of claim 16 wherein said transgenic plant or plant cell is selected from the group of dicotyledonous plants and monocotyledonous plants.

18. (Original) The method of claim 17 wherein said plant cell or transgenic plant is selected from the group of plants including tobacco, rapeseed, soy bean, alfalfa, lettuce, barley, maize, wheat, oat and rice.

19. (Original) The method of claim 1, wherein said CBM fused to said heterologous protein and said CBM fused to said protease are heat-stable and remain soluble at elevated temperatures.

20. (Original) The method of claim 19, wherein one or both of said CBMs are a CBM encoded by a region of the xylanase10A gene from *Thermotoga maritima*.

21. (Currently Amended) The method of claim 20, wherein one or both of said CBMs are encoded by a sequence comprising the sequence shown as SEQ ID NO: 1, or a sequence encoding the same amino acid sequence or an amino acid sequence with at least 80% substantial sequence identity to said sequence.